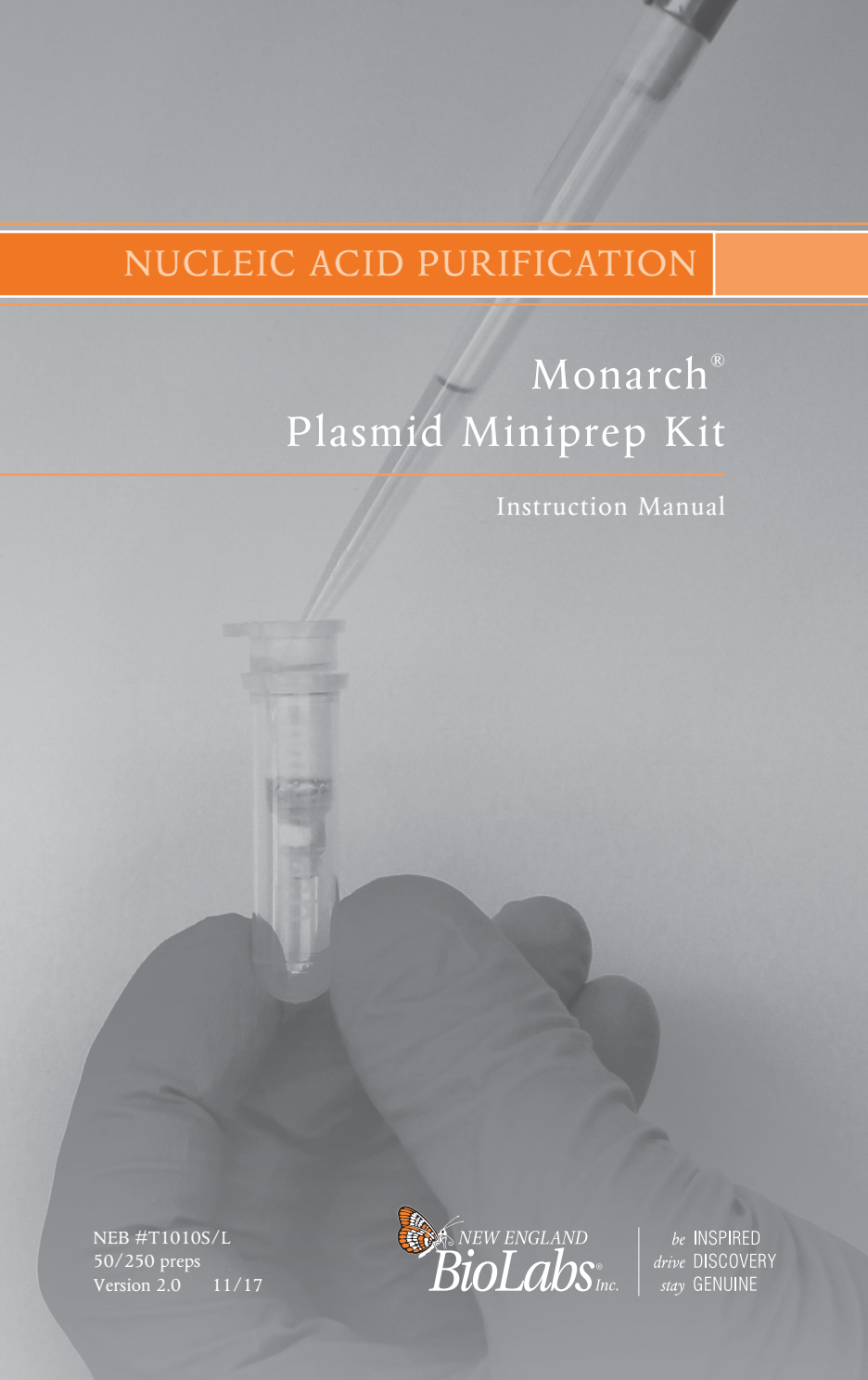


NUCLEIC ACID PURIFICATION

# Monarch®
# Plasmid Miniprep Kit

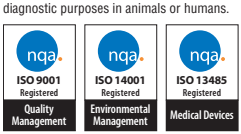
Instruction Manual

NEB #T1010S/L
50/250 preps
Version 2.0 11/17

NEW ENGLAND BioLabs® Inc.

*be* INSPIRED
*drive* DISCOVERY
*stay* GENUINE

This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in animals or humans.



# Monarch Plasmid Miniprep Kit

## Table of Contents:



## Kit Components:

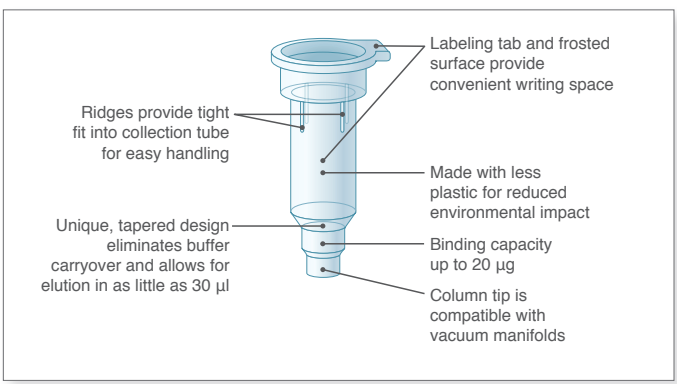
*The kit should be stored at room temperature. Always keep buffer bottles tightly closed and keep columns sealed in the enclosed zip-lock bag. After Plasmid Neutralization Buffer (B3) is opened, it should be stored at 4°C. For information regarding the composition of buffers, please consult the Safety Data Sheets available on our website (www.neb.com/T1010). Proper laboratory safety practices should be employed, including the use of lab coats, gloves, and eye protection.*

| | T1010S 50 preps | T1010L 250 preps | STORAGE TEMP. |
|---|---|---|---|
| Monarch Plasmid Resuspension Buffer (B1) (pink) | 11 ml | 55 ml | Room temp. |
| Monarch Plasmid Lysis Buffer (B2) (blue/green) | 11 ml | 2 x 27 ml | Room temp. |
| Monarch Plasmid Neutralization Buffer (B3) (yellow) | 22 ml | 110 ml | 4°C |
| Monarch Plasmid Wash Buffer 1 | 13 ml | 2 x 27 ml | Room temp. |
| Monarch Plasmid Wash Buffer 2 (5X concentrate) | 6 ml | 30 ml | Room temp. |
| Monarch DNA Elution Buffer | 7 ml | 25 ml | Room temp. |
| Monarch Plasmid Miniprep Columns | 50 | 250 | Room temp. |

# Introduction:

The Monarch Plasmid Miniprep Kit is a rapid and reliable method for the purification of high-quality plasmid DNA. This method employs standard cell resuspension, alkaline lysis and neutralization steps, with the additional benefit of color indicators at each step to easily monitor completion. After clarification of the lysate by centrifugation, the DNA is bound to the proprietary silica matrix under high salt conditions. Unique wash buffers ensure salts, proteins, RNA and other cellular components (endotoxins) are removed, allowing low-volume elution of concentrated, high-purity DNA, ready for use in restriction digests, DNA sequencing, PCR and other enzymatic manipulations.

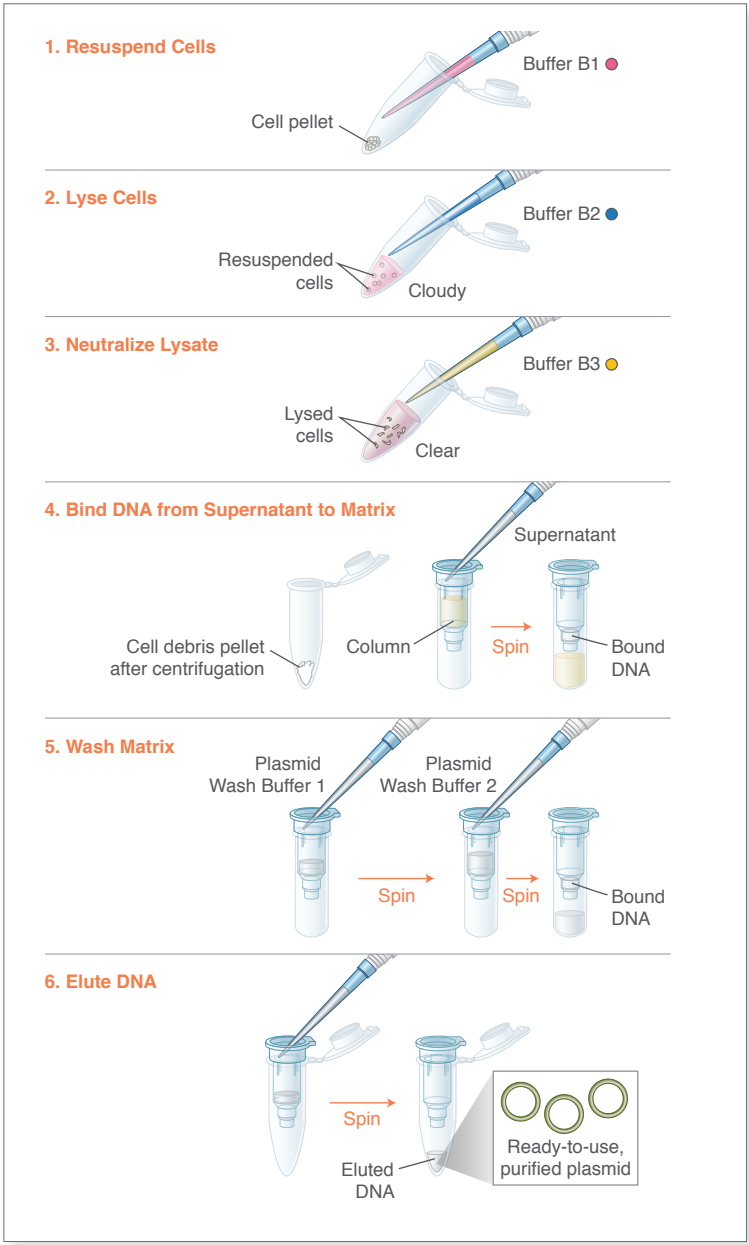
Figure 1: Column design




# Specifications:

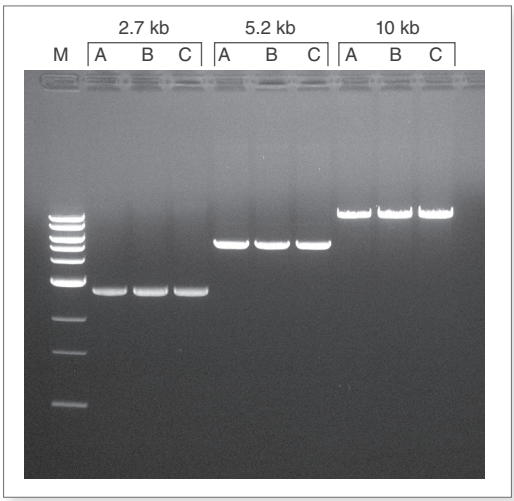
| | |
|---|---|
| **Culture Volume:** | 1–5 ml, not to exceed 15 OD units |
| **Binding Capacity:** | up to 20 µg |
| **Plasmid Size:** | up to 25 kb |
| **Typical Recovery:** | up to 20 µg. Yield depends on plasmid copy number, host strain, culture volume, and growth conditions. |
| **Elution Volume:** | ≥ 30 µl |
| **Purity:** | $A_{260/280}$ and $A_{260/230}$ ≥ 1.8 |
| **Protocol Time:** | 10½ minutes of spin and incubation time |
| **Compatible Downstream Applications:** | restriction digestion and other enzymatic manipulations, transformation, transfection, DNA sequencing, PCR, labeling, cell-free protein synthesis, etc. |

Figure 2: Workflow for plasmid purification



# Performance Data

Figure 3: Monarch Plasmid Miniprep Kit reproducibly generates clean DNA suitable for enzymatic manipulation.



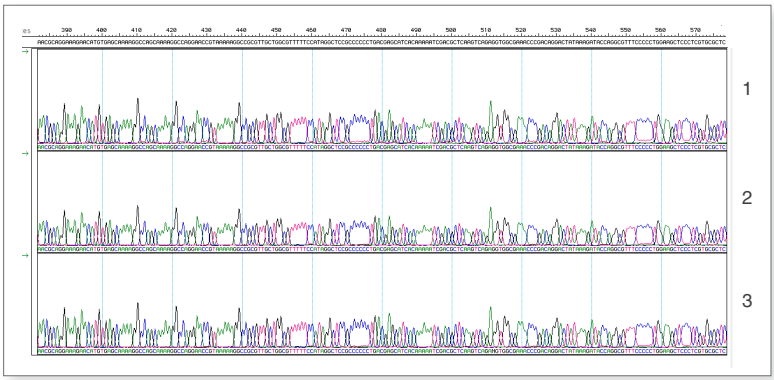

*Equal amounts of three differently sized plasmids produced by the kit were digested in triplicate with a restriction enzyme that recognizes a unique site in each plasmid. Digests were resolved on a 1% agarose gel and stained with ethidium bromide. Digests reproducibly proceed to completion indicating plasmid DNA is high-quality. M is the 1 kb DNA Ladder (NEB #N3232).*

Concentration and purity of triplicate samples of three different size high copy plasmids.

| PLASMID SIZE | CONCENTRATION (ng/ul) | OD 260/280 | OD 260/230 |
|---|---|---|---|
| 2.7 kb | 224.3 +/- 25.0 | 1.91 +/- 0.01 | 2.25 +/- 0.03 |
| 5.2 kb | 255.3 +/- 48.0 | 1.89 +/- 0.02 | 2.26 +/- 0.02 |
| 10 kb | 287.3 +/- 40.5 | 1.89 +/- 0.01 | 2.29 +/- 0.02 |

*Overnight cultures were grown in triplicate from cells harboring one of three differently sized plasmids and 3 ml of each were processed using the Monarch Plasmid Miniprep kit according to the included protocol. Elutions were performed with 50 µl of DNA Elution Buffer and 2 µl of each elution was used to measure concentration and purity with a Nanodrop spectrophotometer. Results are tabulated above and demonstrate yields and purity consistent with expectations including culture to culture variance.*

Figure 4: DNA from Monarch Plasmid Miniprep Kit is reproducibly compatible with DNA sequencing.




*Plasmid DNA from three separate preps was sequenced using BigDye® Terminator chemistry on an Applied Biosystems 3730XL DNA Analyzer. The electropherograms demonstrate the quality of the DNA is reproducible.*

## General Guidelines:

Yield and quality of plasmid DNA is affected by plasmid copy number, plasmid size, insert toxicity, host strain, antibiotic selection, growth media and culture conditions. For standard cloning strains of *E. coli*, we recommend using a single colony from a freshly streaked selective plate to inoculate a standard growth media, such as LB (Luria-Bertaini) media. Cultures are typically grown at 37°C and 200–250 RPM in vessels that allow some aeration (Erlenmeyer flasks or culture tubes on a roller drum) and harvested after 12–16 hours as the culture transitions from logarithmic growth to stationary phase. This is the time at which the plasmid DNA content is highest. While cultures in LB often saturate with a final $OD_{600}$ between 3–6, growth to saturation often leads to cell lysis. As a result, plasmid yields and quality are reduced and the likelihood of co-purifying unwanted host chromosomal DNA increases. Use of rich media, such as 2X YT or TB, produces higher biomass in a shorter time period. If chosen for growth, adjustments to the culture times and amount of cells used in the prep should be made to correct for these differences, and to avoid overloading the matrix and reducing DNA yield and quality.

| PLASMID | REPLICON | COPY NUMBER | CLASSIFICATION |
|---|---|---|---|
| pUC and its derivatives | pMB1* | > 75 | High copy |
| pBR322 and its derivatives | pMB1 | 15–20 | Low copy |
| pACYC and its derivatives | p15A | 10–12 | Low copy |
| pSC101 | pSC101 | ~5 | Very low copy |

*pUC and its derivatives lack the *Rop* gene and contain a point mutation in the RNAII transcript. These changes result in higher copy number during routine growth with many sources reporting levels as high as 500 copies per cell.

Antibiotics for Plasmid Selection

| ANTIBIOTIC | CONCENTRATION OF STOCK SOLUTION | STORAGE TEMP. | WORKING CONCENTRATION |
|---|---|---|---|
| Ampicillin | 100 mg/ml ($H_2O$) | –20°C | 50–200 µg/ml |
| Carbenicillin | 100 mg/ml ($H_2O$) | –20°C | 20–200 µg/ml |
| Chloramphenicol | 34 mg/ml (ethanol) | –20°C | 25–170 µg/ml |
| Kanamycin | 10 mg/ml ($H_2O$) | –20°C | 10–50 µg/ml |
| Streptomycin | 10 mg/ml ($H_2O$) | –20°C | 10–50 µg/ml |
| Tetracycline | 5 mg/ml (ethanol) | –20°C | 10–50 µg/ml |

## Buffer Preparation:

Add ethanol to Monarch Plasmid Wash Buffer 2 prior to use (4 volumes of ≥ 95% ethanol per volume of Monarch Plasmid Wash Buffer 2).

- For 50-prep kit add 24 ml of ethanol to 6 ml of Monarch Plasmid Wash Buffer 2
- For 250-prep kit add 144 ml of ethanol to 36 ml of Monarch Plasmid Wash Buffer 2

Always keep all buffer bottles tightly closed when not actively in use.

## Monarch Plasmid DNA Miniprep Kit Protocol

All centrifugation steps should be carried out at 16,000 x g (~13,000 RPM).

If precipitate has formed in Lysis Buffer (B2), incubate at 30–37°C, inverting periodically to dissolve.

Store Plasmid Neutralization Buffer (B3) at 4°C after opening, as it contains RNase A.

Note: unlike other commercial kits, all wash steps are required.

1. Pellet 1–5 ml bacterial culture (not to exceed 15 OD units) by centrifugation for 30 seconds. Discard supernatant.

   *Note: For optimal performance, be sure to harvest cells at log phase (12-16 hours after inoculation). For a standard miniprep of a high copy plasmid to prepare DNA for restriction digestion or PCR, we recommend 1.5 ml of culture. For low copy plasmids, more culture can be used, but it is important not to process more than 15 OD units per prep/column.*

2. Resuspend pellet in 200 µl Plasmid Resuspension Buffer (B1) ● (pink). Vortex or pipet to ensure cells are completely resuspended. There should be no visible clumps.

3. Lyse cells by adding 200 µl Plasmid Lysis Buffer (B2) ● (blue/green). Invert tube immediately and gently 5–6 times until color changes to dark pink and the solution is clear and viscous. Do not vortex! Incubate for one minute.

*Note: Care should be taken not to handle the sample roughly and risk shearing chromosomal DNA, which will co-purify as a contaminant. Avoid incubating longer than one minute to prevent irreversible plasmid denaturation.*

4. Neutralize the lysate by adding 400 µl of Plasmid Neutralization Buffer (B3) ● (yellow). Gently invert tube until color is uniformly yellow and a precipitate forms. Do not vortex! Incubate for 2 minutes.

   *Note: Be careful not to shear chromosomal DNA by vortexing or vigorous shaking. Firmly inverting the tube promotes good mixing, important for full neutralization.*

5. Clarify the lysate by spinning for 2–5 minutes at 16,000 x g.

   *Note: Spin time should not be less than 2 minutes. Careful handling of the tube will ensure no debris is transferred and the 2 minute recommended spin can be successfully employed to save valuable time. For best results, and especially when using culture volumes > 1 ml, we recommend a 5 minute spin to ensure efficient RNA removal by RNase A. Also, longer spin times will result in a more compact pellet that lower the risk of clogging the column.*

   *To save time, spin for two minutes only.*

6. Carefully transfer supernatant to the spin column and centrifuge for 1 minute. Discard flow-through.

   *To save time, spin for 30 seconds, instead of 1 minute.*

   *If using a vacuum manifold* instead of centrifugation, insert the column into a manifold and switch the vacuum on. Allow the solution to pass through the column, then switch the vacuum source off.*

7. Re-insert column in the collection tube and add 200 µl of Plasmid Wash Buffer 1. Plasmid Wash Buffer 1 removes RNA, protein and endotoxin. (Add a 5 minute incubation step before centrifugation if the DNA will be used in transfection.) Centrifuge for 1 minute. Discarding the flow-through is optional.

   *Note: The collection tube is designed to hold 800 µl of flow-through fluid and still allow the tip of the column to be safely above the top of the liquid. Empty the tube whenever necessary to ensure the column tip and flow-though do not make contact.*

   *To save time, spin for 30 seconds, instead of 1 minute.*

   *If using a vacuum manifold, add 200 µl of Plasmid Wash Buffer 1 and switch the vacuum on. Allow the solution to pass through the column, then switch the vacuum source off.*

** Make sure to follow the manifold manufacturer's instructions to set-up the manifold and connect it properly to a vacuum source.*

8. Add 400 µl of Plasmid Wash Buffer 2 and centrifuge for 1 minute.

   *When using a manifold add 400 µl of Plasmid Wash Buffer 2 and switch the vacuum on. Allow the solution to pass through the column, then switch the vacuum source off.*

9. Transfer column to a clean 1.5 ml microfuge tube. Use care to ensure that the tip of the column has not come into contact with the flow-through. If there is any doubt, re-spin the column for 1 minute before inserting it into the clean microfuge tube.

   *If using a vacuum manifold: Since vacuum set-ups can vary, a 1 minute centrifugation is recommended prior to elution to ensure that no traces of salt and ethanol are carried over to the next step.*

10. Add ≥ 30 µl DNA Elution Buffer to the center of the matrix. Wait for 1 minute, then spin for 1 minute to elute DNA.

    *Note: Nuclease-free water (pH 7–8.5) can also be used to elute the DNA. Delivery of the Monarch DNA Elution Buffer should be made directly to the center of the column to ensure the matrix is completely covered for maximal efficiency of elution. Additionally, yield may slightly increase if a larger volume of DNA Elution Buffer is used, but the DNA will be less concentrated as a result of dilution. For larger plasmids (≥ 10 kb), heating the DNA Elution Buffer to 50°C prior to eluting and extending the incubation time after buffer addition to 5 minutes can improve yield.*

# Troubleshooting

## No DNA Purified

- Plasmid lost during growth of culture. Ensure proper antibiotic is used at correct concentration in order to maintain selection during growth. Do not sub-culture amplicillin-maintained cultures to avoid depletion of antibiotic by secreted β-lactamase. Use a fresh plate and avoid selecting satellite colonies when innoculating the culture.
- Reagents added incorrectly. Check protocol to ensure buffers were added in the correct order and that the sample is bound, washed and eluted in the correct sequence.

## Low DNA Yield

- Incomplete lysis. Ensure cell pellet is completely resuspended before addition of Plasmid Lysis Buffer (B2) and that color changes from light pink to dark pink. Take care to not use too many cells. If culture volume used is larger than recommended, scale-up buffers B1-B3 to ensure proper processing of the sample.
- Plasmid loss during growth, see previous section.
- Low-copy plasmid selected. Increase amount of cells processed and scale buffers accordingly.

- Lysis of cells during growth. Harvest culture during transition from logarithmic growth to stationary phase (typically 12–16 hrs for growth of cultures in LB) to avoid lysis of cells common during extended periods of cell growth.
- Incomplete neutralization. The sample tube should be inverted a sufficient number of times to produce a complete color change to yellow. Cell debris will appear in abundance. Nothing should be floating on the surface after centrifugation, but rather should be compacted into a pellet.
- Incomplete elution. Larger elution volumes and longer incubation times can increase yield of DNA off the column, at the cost of dilution of the sample and increased processing times. For typical plasmids in the 3–10 kb range, the recommended elution volumes and incubation times are sufficient. For the purification of larger plasmids, heating the DNA Elution Buffer to 50°C prior to eluting and extending the incubation time after buffer addition to 5 minutes can improve yield.

### Low DNA Quality

- Plasmid is degraded. Some *E. coli* strains (HB101 and the JM series) have high levels of endogenous endonuclease. Avoid using these when possible. If these strains are used, keep samples on ice during prep and ensure the Plasmid Wash Buffer 1 step is performed.
- Plasmid is denatured. Using Plasmid Lysis Buffer (B2) introduces sodium hydroxide to the DNA. Extended incubation in the presence of sodium hydroxide can separate the strands, or denature the plasmid. Adhere to the protocol and ensure this step is completed within 2 minutes and promptly move on to the neutralization step.
- Plasmid is contaminated with genomic DNA. Vigorous mixing after cell lysis and before pelleting of cell debris may cause shearing of the host cell chromosomal DNA and should be avoided. Additions of Plasmid Lysis Buffer (B2) and Plasmid Neutralization Buffer (B3) should be followed by careful inversion mixing. Do not vortex.
- Improper storage. Ensure DNA is eluted in DNA Elution Buffer or nuclease-free water to maintain integrity and store at –20°C. DNA should not be stored in solutions containing magnesium.

### Low DNA Performance

- Ethanol has been carried-over. Ensure final wash spin time is 1 minute to enable complete removal of the wash buffer from the column. Use care when transferring column to a new tube for the elution step to ensure column tip does not contact column flow-through. If there is any doubt, re-spin the column for 1 minute.
- Excessive salt in sample. Ensure both Plasmid Wash buffers have been utilized according to the protocol. Unlike other commercial kits, all wash steps in the Monarch Plasmid DNA Miniprep Kit protocol are required. All steps should be performed as described to ensure the recovery of high-quality plasmid DNA.

- Excessive carbohydrate carried over. Similar to the endogenous nucleases, strains like HB101 and the JM series, have high amounts of endogenous carbohydrate that can interfere with downstream enzymatic manipulations of plasmid DNA. Be sure to follow the protocol and make sure the Plasmid Wash Buffer 1 step is included.

## Ordering Information

| PRODUCT | NEB # | SIZE |
|---|---|---|
| Monarch Plasmid Miniprep Kit | T1010S/L | 10/50/250 preps |
| **COLUMNS SOLD SEPARATELY** | | |
| Monarch Plasmid Miniprep Columns | T1017L | 100 columns |
| **BUFFERS SOLD SEPARATELY** | | |
| Monarch Plasmid Resuspension Buffer (B1) | T1011L | 55 ml |
| Monarch Plasmid Lysis Buffer (B2) | T1012L | 2 x 27 ml |
| Monarch Plasmid Neutralization Buffer (B3) | T1013L | 110 ml |
| Monarch Plasmid Wash Buffer 1 | T1014L | 2 x 27 ml |
| Monarch Plasmid Wash Buffer 2 | T1015L | 30 ml |
| Monarch DNA Elution Buffer | T1016L | 25 ml |
| **COMPANION PRODUCTS** | | |
| Gel Loading Dye, Purple (6X) | B7024S | 4 ml |
| Gel Loading Dye, Purple (6X), no SDS | B7025S | 4 ml |
| Quick-Load® Purple 1 kb DNA Ladder | N0552S | 125 lanes |
| Quick-Load Purple 100 bp DNA Ladder | N0551S | 125 lanes |
| Quick-Load Purple 2-Log DNA Ladder (0.1 - 10.0 kb) | N0550S | 250 lanes |
| T4 DNA Ligase | M0202S/T/L/M | 20,000/100,000 units |
| Blunt/TA Ligase Master Mix | M0367S/L | 50/250 rxns |
| Instant Sticky-end Ligase Master Mix | M0370S/L | 50/250 rxns |
| **RELATED PRODUCTS** | | |
| Monarch DNA Gel Extraction Kit | T1020S/L | 50/250 preps |
| Monarch PCR & DNA Cleanup Kit (5 µg) | T1030S/L | 50/250 preps |

# How to recycle Monarch Kit components*

| Component | Recycling Notes** |
|---|---|
| **Kit Box** (paper) | For the greatest environmental benefit, please reuse this box. It is fully recyclable in paper recycling. The small magnets do not prohibit recycling. |
| **Columns and Collection Tubes** (hard plastic) | Columns and collection tubes are made from polypropylene 5 and are recyclable. After use, please refer to your institutional policies for proper disposal, especially when working with biohazardous materials. |
| **Plastic Bottles** (hard plastic) | Bottles are made from high-density polyethylene 2, and caps are polypropylene 5. Please rinse before recycling. |
| **Plastic Bags** (plastic film) | Bags are made from low-density polyethylene 4 and can be recycled with other plastic bags and films. |
| **Protocol Card** (paper) | Recycle with mixed paper, or keep in your lab notebook for reference. The finish on this card does not prohibit recycling. |

* Information as of November 2015. Please visit NEBMonarchPackaging.com for updates.
** Please defer to your institutional policies for proper disposal of this kit and its components.
Consult with your local and institutional authorities to learn how to maximize your landfill diversion and materials recovery.

# Citations using the Monarch Plasmid MiniPrep Kit

Luck, A.N., Yuan, X., Voronin, D., Slatko, B.E., Hamza, I., Foster, J.M. (2016). Heme acquisition in the parasitic filarial nematode *Brugia malayi*. *FASEB J.* Jun 30. PubMedID: 27363426 DOI: 10.1096/fj.201600603R. (Epub ahead of print)



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